6. SUMMARY AND CONCLUSIONS

Cadmium is an environmental contaminant adversely affecting a number a tissues with liver and kidney being its major targets. It is a ubiquitous non-essential metal and due to its extraordinarily long biological half life, Cd tends to accumulate in the body. This metal is a serious occupational contaminant and thus may represent a serious health hazard to man and animals. A large number of enzymatic activities are influenced by Cd, which form the basis of Cd toxicity. It promotes early oxidative stress and contributes to the development of serious pathological conditions because of its long retention in some tissues. Different mechanisms have been postulated to explain Cd-induced hepato-renal injury, including increased lipid peroxidation, by depleting GSH or by inhibition of anti-oxidant enzymes. Therefore, protection against the Cd toxicity can be achieved through the supplementation of anti-oxidants.

Eugenol (4-allyl-2-methoxyphenol) is the active component of a number of aromatic plants like basil, cinnamon and bay leaves. It has anti-oxidant, anti-inflammatory, fungicidal and bactericidal properties. Eugenol is also known to possess anti-genotoxic and anti-carcinogenic activity. The effect of eugenol to combat Cd toxicity was studied in the present work.

The hepato-renal acute toxicity of Cd and its modulation by eugenol was evaluated in male BALB/c mice. For this a multiparametric approach including biochemical, morphological, ultrastructural and genotoxic parameters on liver and kidney tissues was employed. For studying time dependent acute Cd toxicity, 5 mg/kg b.wt. Cd (CdSO₄) was given i.p. for one, three and five days. Pre-, concurrent and post-treatments of two eugenol doses dissolved in DMSO (5 mg/kg b.wt. and 10 mg/kg b.wt. for five days) was given to study the beneficial effects of eugenol on Cd mediated toxicity.
The results of present study are summarized as follows:

- Lipid peroxidation, a deleterious process solely carried out by free radicals is considered as the primary mechanism for Cd toxicity. Marked elevation of hepatic and renal LPO after one, three and five days of Cd administration was observed in the present study. After five days, approximately two fold increase in hepato-renal LPO was seen.

- The impairment of the anti-oxidant defense system is considered as a critical event in Cd-induced hepatic and nephro-toxicity. One, three and five days Cd exposure caused a marked fall in the level of reduced glutathione. Approximately 50% decline was observed in GSH levels after five days of Cd treatment. In the present study, decreased levels of GSH following Cd treatments (5 mg/kg b.wt.) might increase the susceptibility of the respective tissues to free radical damage.

- Cadmium toxicity declines SOD and CAT activities, which mutually constitute a supportive team of defense against ROS. In the present study one day Cd (5 mg/kg b.wt.) administration caused insignificant decrease in SOD and increase in CAT levels in hepatic and renal tissues. Three and five days Cd treatment (5 mg/kg b.wt.) resulted in marked decrease in SOD and CAT hepato-renal levels. The observed decrease in the activities of SOD and CAT may either be due to the direct binding of the metal to the active site of the enzymes or due to their increased usage in scavenging free radicals induced by the metal.

- One day Cd (5 mg/kg b.wt.) treatment caused slight increase in the levels hepato-renal GST and GR in mice, whereas their activities were reduced significantly in three and five days Cd treatment. The formation of Cd sulfhydryl complex with -SH groups of enzyme might decrease the activities of GR and G6PD and depletion of GSH levels making the cells more susceptible to toxic electrophilic compounds.
Liver and kidney injury followed by Cd exposure was well established by the elevated levels of serum hepatic and renal function marker enzymes indicating cellular leakage and loss of functional integrity of hepatic membrane architecture. Present study revealed increased activities of AST, ALT, ALP, bilirubin, urea and creatinine in the serum of five days Cd-treated (5 mg/kg b.wt.) mice and suggested that an extensive tissue damage had occurred probably due to increased lipid peroxidation. Cd caused structural and functional damage to the cell membrane and increased the membrane permeability leading to the leakage of hepatic and renal functional markers into the blood.

In the present study, the hepatic histo-architecture of the Cd-treated mice (three and five days) showed severe necrotic changes, inflammatory cell infiltration, fatty degeneration and vacuolization. Ultrastructural alterations like enlargement of Disse space, sinusoidal collapsing, distortion of endothelial cells, dilation of bile canaliculi, loss of microvilli from the hepatocyte surface, fat deposition and marked peliosis were revealed by SEM, whereas TEM exhibited proliferation of SER, loss of ribosomes from the RER, destruction of RER, appearance of secondary lysosomes, the presence of lipid droplets and mitochondrial swelling and loss of cristae in Cd treated (5 mg/kg b.wt. for five days) mice liver. These changes might occur due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by Cd. The accumulated hydroperoxides may cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxides, the basis for hepatocellular damage. The necrotic conditions coincide with our biochemical observations, which showed the increased level of lipid peroxidation following Cd exposure.

The histopathological examination of Cd-treated mice kidney showed glomerular shrinkage, increase in mesengial space, vascular congestion, protein cast in the PCT and DCT, tubular
necrosis, inflammatory cell infiltration, tubular degeneration, hemorrhage and swelling of tubules. SEM of kidney revealed shrunked glomeruli in Bowman’s capsule, increased mesengial space, swelling in podocytes and irregular foot processes, where as transmission electron micrograps of Cd treated kidney (5 mg/kg b.wt. for five days) demonstrated ultrastructural changes including irregular or absence of brush-border lining the tubules, nuclear degeneration, condensation, mitochondrial swelling, degradation and dilation of rough endoplasmic reticulum, increase in lysosomes and hydropic vacuoles and presence of myelin figures in the tubular epithelium. The renal damage may be due to the Cd-MT complex generated in the liver which is released in the plasma and then filtered through the glomeruli in the kidney and taken up by the proximal tubular cells hence causing damage in renal tissues.

- Bone marrow chromosomal analysis of Cd treated groups clearly exhibited the clastogenic and genotoxic effect of Cd. Cd significantly increased the frequency of chromosome aberrations, particularly; single chromatid break, multiple chromatid break, ring formation, centromeric fusion, centromeric separation and Y chromosome break in bone marrow cells. Present study has suggested that ROS are involved in DNA damage induced by clastogenicity of metal ions like Cd$^{2+}$. Cd triggers the chromosomal aberrations by induction of LPO and inhibition of GSH-mediated, GST-catalyzed detoxification and hence producing reactive metabolites that can interact with the chromatin material.

- In the present study, eugenol (5 mg/kg b.wt., 10 mg/kg b.wt.) administration lowered the LPO levels in Cd treated group. Pre-treatment of higher eugenol (10 mg/kg b.wt.) dose was most effective in lowering LPO levels. It may be due to its property to chelate metal ions and decrease the formation of hydroxyl radical by inhibiting metal dependent fenton reaction. Pre-treatment of
eugenol showed better results than the concurrent and post-treatments.

- Administration of eugenol (pre-treatment 10 mg/kg b.wt.) increased hepato-renal SOD and CAT activities that might be due to its ability to reduce the accumulation of free radicals generated during Cd-induced lipid peroxidation.

- Administration of eugenol (pre-treatment 10 mg/kg b.wt.) along with Cd significantly elevated the levels of glutathione and glutathione-metabolizing enzymes (GST and GR). The abilities of eugenol to react with highly reactive by-products (produced by lipid peroxidation) and enhancement of tissue thiol pools may cause the restoration of the activities of anti-oxidant enzymes and glutathione-metabolizing enzymes.

- Eugenol pre-treatment (10 mg/kg b.wt.) attenuated Cd-induced hepatic and nephrotoxicity as seen by the decreased levels of AST, ALT, ALP, bilirubin, and reduced serum levels of urea and creatinine thus offering protection against Cd toxicity in mice. Eugenol exerts its chemopreventive potential due to its phenolic group that may offer protection by stabilizing the cell membrane in hepato-renal damage induced by Cd.

- Pre-treatment of eugenol (10 mg/kg b.wt.) reduced the histological alterations induced in liver and kidney by Cd (5 mg/kg b.wt. for five days) quite appreciably. It could be attributed to the anti-radical/anti-oxidant and metal-chelating efficacy of eugenol which significantly reduced the oxidative stress leading to the reduction of histo-pathological alterations and restoration of normal physiological state of an organism. The decomposition products of lipid hydroperoxides can cause chaotic cross linkage with proteins and nucleic acids which plays an important role in Cd-induced hepatic and renal toxicity. Therefore, a plausible mechanism could be that eugenol has inhibitory effect on inflammatory mediators and thus exhibited its protective effect against Cd induced ischemia and secondary inflammatory reactions.
Eugenol pre-treatment (10 mg/kg b.wt.) had a protective effect against the Cd-induced genotoxicity and markedly reduced the chromosomal aberrations. Eugenol might directly detoxify the reactive metabolites by donating its electrons and thus reduced the Cd induced genotoxicity.

It can thus be summarized that the administration of eugenol in Cd-intoxicated animals counteracted the oxidative hepato-renal dysfunction attributed by Cd. Pre-treatment with eugenol (10 mg/kg b.wt.) appreciably reduced the abnormal changes induced by Cd and more effectively restored the biomarkers of oxidative stress and hepato-renal toxicity towards near normal than the lower dose (5 mg/kg b.wt.) of eugenol and even the concurrent and post-treatments of the same dose (10 mg/kg b.wt.). This was observed as restoration in the levels of serum hepatic and renal function markers, increase in the activities of anti-oxidant enzyme cascade, improvement in the levels of non-enzymatic anti-oxidants along with the decreased levels of LPO in liver and kidney.

Protective action of eugenol for tissue architecture following Cd exposures was supported by the improvement in the histopathological changes induced by Cd in hepatic and renal tissues. Anti-clastogenic potential of the eugenol was further evidenced by reducing the chromosomal aberration aggravated by Cd intoxication. In view of the present study, it can be deduced that eugenol (pre-treatment 10 mg/kg b.wt.) played a role of an anti-oxidant which includes free radical scavenging and metal-chelating property and thereby improved the detrimental state of liver, kidney and bone marrow cells following Cd exposures unravel the use of eugenol as a possible mitigator/attenuating agent in Cd-induced toxicity.

From the present work, it can be concluded that eugenol pre-treatment (10 mg/kg b.wt.) significantly curtails the toxic effects of Cd on liver and kidney, suggesting that eugenol could be considered as a potential drug supplement for preventing hepato-renal damage induced by metal toxicity.